76 Regulation of the Neurotransmitter Norepinephrine

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ABSTRACT The catecholamines dopamine, norepinephrine, and epinephrine are in a state of flux, yet they maintain a constant level in nerves and glandular tissues. The level of these biogenic amines is regulated by changes in activity of the biosynthetic enzymes tyrosine hydroxylase, dopamine-β-hydroxylase, and phenylethanolamine-N-methyltransferase. The minute-to-minute regulation of the level of the neurotransmitter norepinephrine is controlled by rapid changes in tyrosine hydroxylase activity caused by feedback inhibition of the enzyme by norepinephrine and dopamine. There is no change in the amount of enzyme protein. Elevation in tyrosine hydroxylase occurs in the cell body, nerve terminals, and adrenal medulla when there is an increase in firing of sympathetic nerves. This results in formation of new enzyme protein by a transsynaptic process. A similar transsynaptic induction by increased nerve firing occurs with the enzyme dopamine-β-hydroxylase in nerves and adrenal medulla. The induction of these enzymes appears to be initiated by acetylcholine and possibly controlled by intracellular concentrations of norepinephrine. The activity of tyrosine hydroxylase and dopamine-β-hydroxylase and especially phenylethanolamine N-methyltransferase in the adrenal medulla is reduced by removal of the pituitary gland and induced by ACTH.

Dopamine-β-hydroxylase is transported from cell body to nerve terminals. When nerves are depolarized, dopamine-β-hydroxylase is released from the nerves, together with the neurotransmitter norepinephrine, by a process of exocytosis. The release of dopamine-β-hydroxylase requires Ca^{++}, microtubules, and microfilaments.

The biogenic amine serotonin undergoes a circadian change in levels in the pineal gland. The level of serotonin is regulated by the neurotransmitter norepinephrine released from sympathetic nerves. Norepinephrine reduces the serotonin levels by stimulating the enzyme that acetylates serotonin via cyclic AMP. Changes in the rate of neuronal release of norepinephrine markedly influence the activity of N-acetyltransferase.

THE ACTIVITY OF the sympathetic nervous system undergoes rapid changes yet maintains a constant level of its neurotransmitter, norepinephrine. This is made possible by a variety of self-regulatory systems involving changes in its biosynthesis, storage, release, and metabolism within the neuron as well as modifications of the pre- and post-

synaptic membrane. The special morphology of the sympathetic neuron also contributes to the maintenance of the neurotransmitter. The sympathetic neuron consists of a cell with a considerable spatial separation from its nerve terminals (Figure 1). The nerve terminals are highly branched and have swellings or varicosities that are in close proximity to the effector cells. Within the varicosity, norepinephrine is stored in a dense core vesicle of about 500 Å (Wolfe et al., 1962). This structural organization is present in both the peripheral and central nervous systems. The cell body of a sympathetic neuron synapses with a preganglionic fiber, usually cholinergic, and the varicosity of the nerve terminals innervates many thousand effector cells *en passant*.
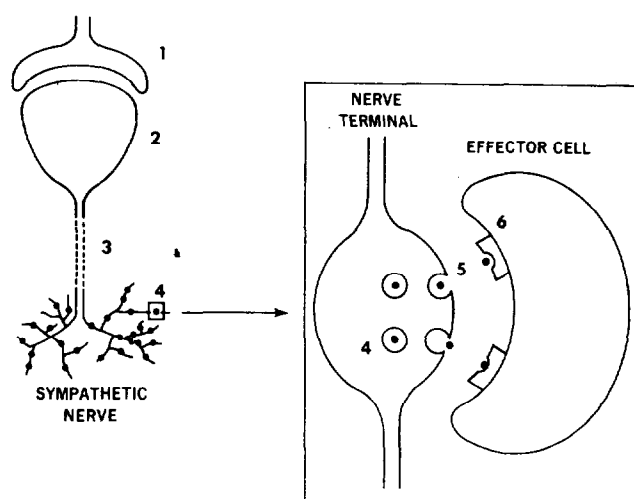

FIGURE 1 Sites at which the neurotransmitter norepinephrine can be regulated (see text for explanation).

The levels of norepinephrine within the sympathetic neuron can be regulated at several sites (Figure 1): Cell body via preganglionic nerves (areas 1, 2); the axon, which transports the biosynthetic enzymes made in the cell body (area 3); the cytoplasm and storage vesicle of nerve terminal (area 4); the neuronal membrane (area 5); and the postsynaptic membrane (area 6).

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Biosynthesis of norepinephrine and epinephrine

The enzymes involved in the formation of norepinephrine are synthesized in the cell body of the sympathetic neuron. These enzymes are tyrosine hydroxylase, which converts tyrosine to dopa (Nagatsu et al., 1964), dopa decarboxylase (Holtz et al., 1938), and dopamine-β-hydroxylase, the enzyme that β-hydroxylates dopamine to norepinephrine (Friedman and Kaufman, 1965). Phenylethanolamine-N-methyltransferase, the enzyme that methylates norepinephrine to epinephrine, is present mainly in adrenal medulla of mammals and sympathetic nerves of amphibians (Axelrod, 1962). Tyrosine hydroxylase is a mixed function oxidase requiring tetrahydropteridine and O_2 and Fe^{++}. It is found in the cell body, axon, nerve terminals, as well as the adrenal medulla, and is absent in extraneural tissue. Tyrosine hydroxylase is found in soluble and bound form. A molecular weight of 192,000 has been reported for the soluble enzyme, while the bound enzyme after trypsin digestion has a molecular weight of 50,000 (Wurzburger and Musacchio, 1971). Tyrosine hydroxylase can be inhibited by norepinephrine, which serves as an important controlling mechanism for its synthesis. Inhibition of the enzyme by catecholamines is competitive for its pteridine cofactor in its reduced form and not with its substrate, tyrosine (Ikeda et al., 1966). Dopa decarboxylase is unspecific in that it can decarboxylate a variety of L-aromatic amino acids. It requires pyridoxal phosphate as a cofactor and is tightly bound to the apoenzyme as a Schiff base. Dopa decarboxylase is present both in neuronal and extraneuronal tissues. Using an immunoassay, it was shown that aromatic acid decarboxylase is a single enzyme with a molecular weight of 109,000 (Christenson et al., 1972). Dopamine-β-hydroxylase hydroxylates dopamine on the beta carbon to form norepinephrine. It is a mixed function oxidase containing

2 mole of Cu^{++}, which is reduced by ascorbic acid (Friedman and Kaufman, 1965). The enzyme lacks specificity and can β-hydroxylate a variety of phenylethylamines. The enzyme is highly localized in the sympathetic neuron as well as the adrenal medulla. Within the neuron dopamine-β-hydroxylase is present in the cell body, axon, and nerve terminal. It is highly localized in the norepinephrine storage vesicles of nerves (Potter and Axelrod, 1963a) and chromaffin granules of the adrenal medulla (Kirshner, 1957).

The epinephrine-forming enzyme, phenylethanolamine-N-methyltransferase, is highly localized in the cytoplasm of mammalian adrenal medulla (Axelrod, 1962) and is present in sympathetic nerves of amphibians (Wurtman et al., 1968b). It methylates norepinephrine as well as β-hydroxylated phenylethanolamine derivatives; S-adenosylmethionine is the methyl donor. The enzyme has been purified and its molecular weight has been found to be about 30,000 (Connett and Kirshner, 1970). Phenylethanolamine-N-methyltransferase shows different electrophoretic mobility on starch block, and multiple forms of the enzyme have also been reported (Axelrod and Vesell, 1970). The biosynthesis of catecholamines is shown in Figure 2.

Neural regulation of the catecholamine biosynthetic enzymes

In a study to determine the intraneural localization of tyrosine hydroxylase, 6-hydroxydopamine, a compound that destroys sympathetic nerve terminals (Thoenen and Tranzer, 1968), was administered to rats. There was an almost complete disappearance of this enzyme in the heart in 40 hr suggesting that it was highly localized in sympathetic nerve terminals (Mueller et al., 1969a). When tyrosine hydroxylase was measured in the adrenal gland
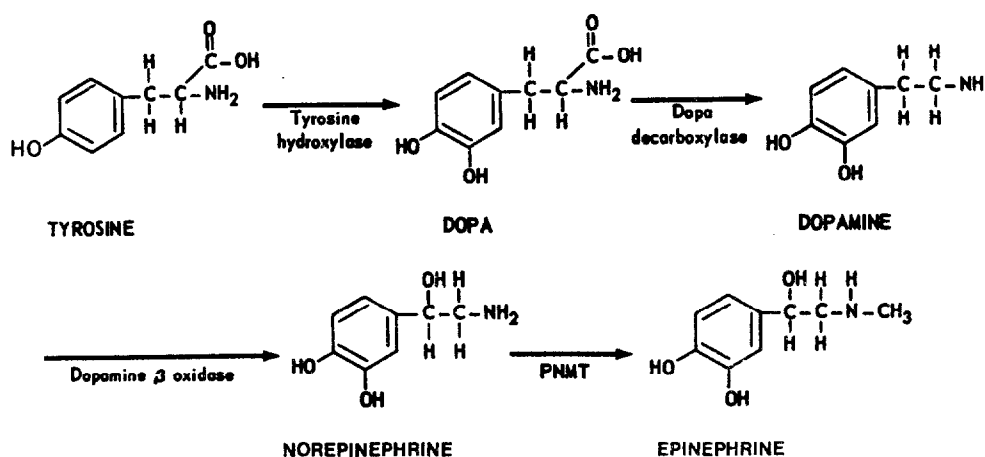
Figure 2   Biosynthesis of catecholamines. PNMT is phenylethanolamine N-methyltransferase.

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there was a marked increase in this enzyme and a smaller elevation of phenylethanolamine-N-methyltransferase about 1 day after the administration of 6-hydroxydopamine. This was an unexpected finding, and it appeared to be due to the ability of 6-hydroxydopamine to lower blood pressure. This would cause a reflex increase in sympathetic nerve activity, resulting in an increase in enzyme activity in the adrenal gland. To examine this possibility, reserpine and phenoxybenzamine, compounds that lower blood pressure and increase sympathetic nerve activity, were given, and their effects on tyrosine hydroxylase in the adrenal gland examined (Mueller et al., 1969b). Both compounds elevated tyrosine hydroxylase activity, not only in the adrenal gland but also in the superior cervical (Figure 3) and stellate ganglia. The maximal enzyme activity observed in the adrenal gland and the ganglia occurred 3 days after reserpine administration, indicating a slow rise in enzyme activity. Reserpine was also found to increase tyrosine hydroxylase activity in adrenal gland of all mammalian species examined as well as the brainstem of the rabbit. Increased tyrosine hydroxylase activity after reserpine was also observed in the nerve terminal as well as in the cell body. The increase in enzyme activity in the nerve terminal was delayed and lagged behind the rise in the ganglia by 2 days (Thoenen et al., 1970).

The elevation in tyrosine hydroxylase activity after the increase in sympathetic nerve activity was shown physiologically by the increased formation of [^{14}C]catechol-amine from [^{14}C]tyrosine after the administration of phenoxybenzamine or 6-hydroxydopamine (Dairman and Udenfriend, 1970; Mueller, 1971). The rise in tyrosine hydroxylase activity in the ganglia and adrenal medulla after reserpine administration could be prevented by the administration of cycloheximide or actinomycin D (Mueller et al., 1969c), suggesting that this elevation of enzyme activity is due to induction of new enzyme protein. The Km for both the substrate and the pteridine cofactor with the enzyme obtained from reserpine-treated rats was not different from the untreated rats, although there was a marked elevation in the V_{max} for both substrate and cofactor. These results are consistent with an increase in the number of active sites on the enzyme molecule caused by a drug-induced rise in sympathetic nerve activity. Increased tyrosine hydroxylase activity was found in the adrenal gland after cold (Thoenen et al., 1969a), immobilization (Kvetnansky et al., 1970), and psychosocial stress (Axelrod et al., 1970), in the ganglia after administration of nerve growth factor (Thoenen, 1970), and in brain after cold stress and administration of reserpine (Segal et al., 1971).

To examine whether the induction in tyrosine hydroxylase after reserpine was a transsynaptic event the preganglionic fibers to the superior cervical ganglia (Thoenen et al., 1969a) and the splanchnic nerve to the adrenal gland were cut unilaterally (Thoenen et al., 1969b). When reserpine was administered, there was an elevation
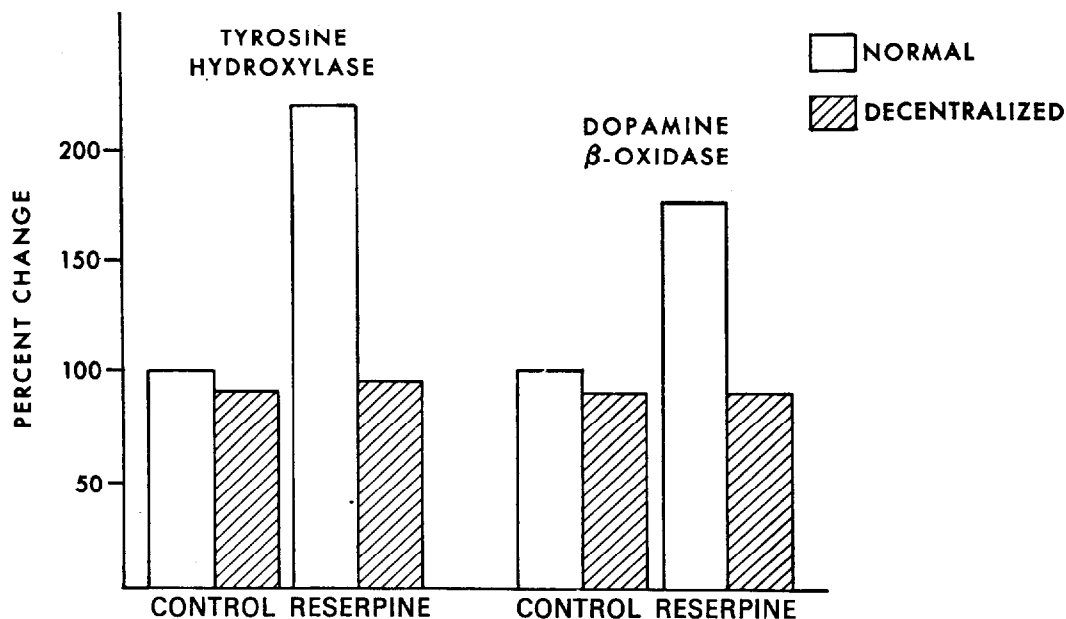

FIGURE 3 Transsynaptic induction of tyrosine hydroxylase and dopamine-β-oxidase(hydroxylase) in sympathetic ganglia. Superior cervical ganglion was decentralized unilaterally by transection of the preganglionic trunk. Two to six days later reserpine (5 mg/kg) was given for 1 day before tyrosine hydroxylase was measured or 3 alternate days before dopamine-β-oxidase(hydroxylase) was measured (from Axelrod, 1971).

of enzyme activity in the innervated side of the ganglia and adrenal gland, but the increase on the denervated side was completely blocked (Figure 3). All of these observations indicate that tyrosine hydroxylase activity is increased in both the sympathetic nerve, cell body, brain, and adrenal medulla by sympathoadrenal hyperactivity. This elevation in tyrosine hydroxylase activity appears to involve a transsynaptic induction of new enzyme molecules.

The presynaptic fibers that regulate tyrosine hydroxylase activity are cholinergic. Interrupting the cholinergic splanchnic nerve blocks the drug-induced rise in tyrosine hydroxylase (Thoenen et al., 1969a, 1969b). Ganglionic blocking agents also inhibit the increase in this enzyme (Mueller et al., 1970b), while acetylcholine causes an elevation of tyrosine hydroxylase activity in the adrenal (Patrick and Kirshner, 1971). In the superior cervical ganglia of the newborn mouse, the development of tyrosine hydroxylase is prevented by cutting the preganglionic cholinergic nerve (Black et al., 1971), suggesting that presynaptic cholinergic terminals regulate the formation of tyrosine hydroxylase in the sympathetic nerve cell body. The selective destruction of sympathetic nerves chemically with 6-hydroxydopamine or immunologically with antiserum to the nerve growth factor prevents the normal development of choline acetyltransferase in presynaptic nerve endings, indicating that postsynaptic adrenergic neurons regulate the biochemical development of presynaptic cholinergic nerves (Black et al., 1971).

Breeding studies are being carried out utilizing reciprocal F_1 and F_2 and dominant recessive backcross generations with respect to the catecholamine biosynthetic enzymes (Ciaranello, unpublished observations). Preliminary results suggest that the genes controlling tyrosine hydroxylase, dopamine-β-hydroxylase and phenylethanolamine-N-methyltransferase are linked. It is possible that the three genes are linked and that a single regulatory locus is responsible for the activity of the three biosynthetic enzymes.

The activity of dopamine-β-hydroxylase is also affected by nerve impulses. The development of a sensitive assay for measuring dopamine-β-hydroxylase made it possible to measure this enzyme in the cell body and nerve terminals and to study changes after drugs that increase sympathetic nerve firing (Molinoff et al., 1971). The administration of reserpine resulted in a marked elevation of dopamine-β-hydroxylase activity in sympathetic ganglia (Figure 3), nerve terminals, and adrenal gland but not in the brain (Molinoff et al., 1970). This increase in enzyme activity in cell bodies is neuronally mediated, because the reserpine could not elevate dopamine-β-hydroxylase activity in a denervated ganglia (Figure 3). Pretreatment of animals with the protein synthesis in-

hibitor, cycloheximide, prevented the rise in enzyme activity in the ganglia. Further evidence that new enzyme protein was induced by nerve impulses comes from the use of an antibody for dopamine-β-hydroxylase. Reserpine caused an increase in the rate of incorporation of [^{3}H]leucine into dopamine-β-hydroxylase measured by immunoabsorption (Hartman et al., 1970).

It appears that nerve depolarization is involved in the induction of dopamine-β-hydroxylase. An increase in the potassium concentration of the media containing rat superior cervical ganglia maintained in organ culture results in a marked increase in dopamine-β-hydroxylase in ganglia (Silberstein et al., 1972). This increase in enzyme activity is inhibited by cycloheximide. Nicotinic antagonists block the induction of dopamine-β-hydroxylase in ganglia after reserpine, suggesting that a cholinergic site is involved (Molinoff et al., 1972). Acetylcholine also increases dopamine-β-hydroxylase activity in the denervated adrenal gland (Patrick and Kirshner, 1971). However, it does not appear that the cholinergic receptor is essential for induction of the enzyme, at least in sympathetic nerves, because elevated potassium concentration can increase enzyme activity in the absence of neuronal influences.

Another biosynthetic enzyme, phenylethanolamine-N-methyltransferase, in the adrenal gland, is regulated by neuronal influences. Increasing splanchnic nerve activity with 6-hydroxydopamine, reserpine, or stress causes a small elevation of phenylethanolamine-N-methyltransferase in the rat adrenal gland (Mueller et al., 1969a) and a large increase in the mouse adrenal (Ciaranello et al., 1972a). This increase can be abolished by transection of the nerve supplying the adrenal gland (Thoenen et al., 1970).

Unlike the other catecholamine biosynthetic enzymes, dopa decarboxylase activity in the superior cervical ganglia or adrenal gland is not induced by drug-mediated increase in preganglionic neural activity (Black et al., 1971). These experiments suggest that tyrosine hydroxylase, dopamine-β-hydroxylase, and phenylethanolamine-N-methyltransferase are linked in a coordinate fashion. Genetic studies also indicate that these enzymes are linked (Ciaranello, unpublished observations).

Several experiments suggest that catecholamines are implicated in the induction of dopamine-β-hydroxylase and tyrosine hydroxylase (Molinoff et al., 1972). Drugs that elevate the level of catecholamines, such as L-dopa, monoamine oxidase inhibitors, and bretylium, inhibit the induction of both tyrosine hydroxylase and dopamine-β-hydroxylase. On the other hand, reduction of catecholamines by α-methyl-paratyrosine or high potassium (Silberstein et al., 1972) results in an induction of dopamine-β-hydroxylase activity.

The nerve terminal may have an important influence on the induction of the catecholamine biosynthetic enzymes in the cell body. Destruction of adrenergic nerve terminals with 6-hydroxydopamine or surgical section of the postganglionic axons causes a long-lasting decrease in dopamine-β-hydroxylase in the superior cervical ganglia (Brimijoin and Molinoff, 1971). 6-Hydroxydopamine administration or a postganglionic section also results in a marked increase in the uptake of [^{3}H]norepinephrine in the sympathetic ganglia (Kopin and Silberstein, 1972). The latter phenomena reflects growth of adrenergic membrane surface. These experiments show that when nerve terminals are destroyed the metabolic machinery of the cell body changes its priorities from the production of enzymes concerned with function to the formation of structural elements required for the restoration of the nerve ending.

Hormonal regulation

In addition to nervous inputs, the corticoids can also influence the biosynthesis of catecholamines. The effects of hormones are principally in the adrenal medulla, a structure that can be considered analogous to the cell body of the sympathetic nervous system. An examination of the effects of hormones on catecholamine formation was prompted by the observation that large amounts of epinephrine are present in the adrenal gland of those species in which the medulla is surrounded by a cortex (Coupland, 1965). This suggested to us that corticoids

present in the cortex might be the compounds that stimulate the methylation of norepinephrine to epinephrine. The experimental design to examine this possibility was to reduce corticoids in adrenal cortex by removal of the pituitary gland in rats and then to measure the activity of phenylethanolamine-N-methyltransferase in the adrenal gland (Wurtman and Axelrod, 1966). When rats were hypophysectomized, there was a gradual and steady decline in the norepinephrine methylating enzyme. After about 7 days, only about 20% of the enzyme activity remained in the adrenal medulla (Figure 4). The administration of either dexamethasone, a potent glucocorticoid, or ACTH restored phenylethanolamine-N-methyltransferase activity to the adrenal gland after several days (Figure 4). Inhibition of protein synthesis blocked the increase in enzyme activity after the administration of dexamethasone. When dexamethasone or ACTH was given repeatedly to normal rats there was no increase in enzyme activity in the adrenal gland. All of these experiments demonstrated that glucocorticoids in the adrenal cortex are necessary to maintain phenylethanolamine-N-methyltransferase activity. There are negligible amounts of phenylethanolamine-N-methyltransferase in sympathetic nerve cell body. When dexamethasone is given to newborn rats, phenylethanolamine-N-methyltransferase appears in the superior cervical ganglia (Ciaranello, Jacobowitz, and Axelrod, unpublished observations). The ability of dexamethasone to induce the methylating enzyme in the ganglia is lost after the rat is 2 weeks old. Dexamethasone also increased
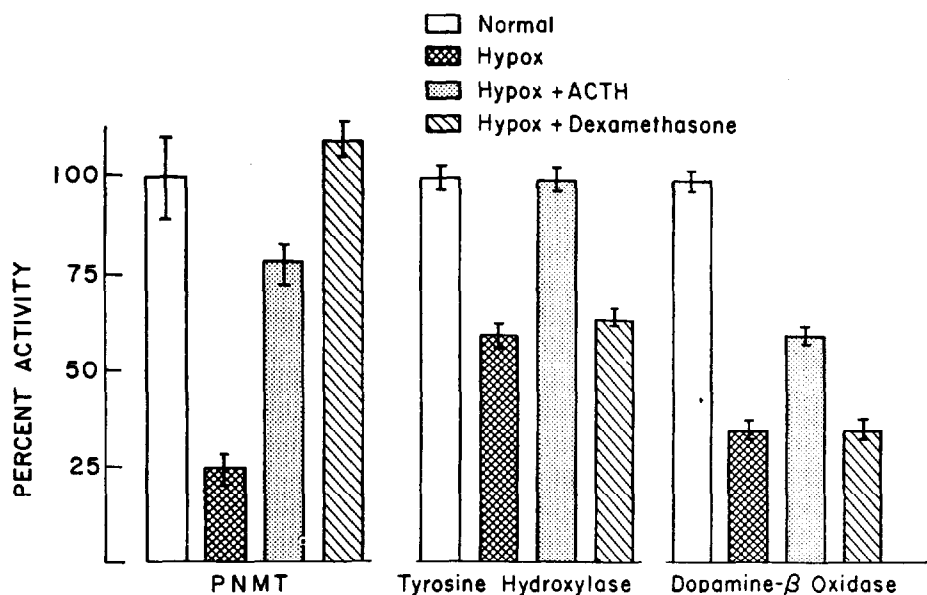

FIGURE 4 Hormonal regulation of catecholamine biosynthetic enzymes in the adrenal. Rats were hypophysectomized for about 1 week and then given dexamethasone or ACTH (from Axelrod, 1973).

the amounts of small, intensely fluorescent (SIF) cells in the superior cervical ganglia. These cells are morphologically related to chromaffin cells. These findings indicate that glucocorticoid hormones may be involved in differentiation of nerve cell to chromaffin type cell .

Removal of the pituitary also affected other catecholamine biosynthetic enzymes in the adrenal medulla. After rat hypophysectomy the activity of tyrosine hydroxylase (Mueller et al., 1970a) and dopamine-β-hydroxylase fell (Weinshilboum and Axelrod, 1970) (Figure 4). Repeated administration of ACTH restored the activity of both enzymes in the adrenal gland (Figure 4). However, dexamethasone failed to elevate tyrosine hydroxylase or dopamine-β-hydroxylase (Figure 4). When mice were subjected to psychosocial stimulation, there was marked elevation of tyrosine hydroxylase and phenylethanolamine-N-methyltransferase in the adrenal gland (Axelrod et al., 1970). When certain mouse strains were exposed to cold stress for 3 to 6 hr, there was a small but significant elevation of phenylethanolamine-N-methyltransferase in the adrenal (Ciaranello et al., 1972a). Implantation of an ACTH-secreting tumor in rats resulted in an elevation of phenylethanolamine-N-methyltransferase, demonstrating that the enzyme can be elevated under conditions of extreme pituitary-adrenocortical activation. Forced immobilization stress also increases tyrosine hydroxylase and dopamine-β-hydroxylase in adrenal to a considerable extent and phenylethanolamine-N-methyltransferase to a smaller degree (Kvetnansky et al., 1970). These stress-induced elevations are mediated by neuronal and hormonal influences.

Regulation of catecholamines at nerve terminals

There is a rapid regulation of the biosynthesis of the adrenergic neurotransmitter in the nerve terminals, which is different from the slower induction of the catecholamine-forming enzymes described above. Stimulation of the splanchnic nerve leads to a release of catecholamines. The sum of the amount of catecholamines released and that remaining in the gland is greater than the amount initially present in the gland (Bydgeman and von Euler, 1958). Studies with the hypogastric nerve (Weiner, 1970) and salivary gland (Sedvall and Kopin, 1967) indicate that the rapid changes in the biosynthesis of norepinephrine are regulated by tyrosine hydroxylase. Stimulation of the sympathetic nerve of the vas deferens in vitro or the salivary gland in vivo (Table I) led to an increased conversion of [^{14}C]tyrosine to [^{14}C]norepinephrine. However, there was no increase in the formation of [^{14}C]dopa to [^{14}C]norepinephrine when the nerves were stimulated, suggesting that tyrosine hydroxylase is the enzyme in-

fluenced by nerve activity. However, there was no increase in the amount of tyrosine hydroxylase in the stimulated salivary gland. In an in vitro study with the vas deferens, it was found that addition of norepinephrine to the bath can partially or completely prevent the increased formation of [^{14}C]norepinephrine from [^{14}C]tyrosine (Weiner, 1970). It has been shown that tyrosine hydroxylase is inhibited by catecholamines such as dopamine and norepinephrine, due to the competition between the catecholamines and the pteridine cofactor (Ikeda et al., 1966). Most of the norepinephrine in the nerve terminals is present in vesicles with little access to tyrosine hydroxylase that appears to be present in the cytoplasm. Thus, there is a small compartment of free catecholamines in the cytoplasm that is critical in the regulation of tyrosine hydroxylase. This small compartment is rapidly depleted during nerve stimulation and thus allows more norepinephrine to be synthesized by increasing the conversion of tyrosine to dopa.

TABLE I

Effect of sympathetic nerve impulses on norepinephrine synthesis and tyrosine hydroxylase activity in the rat salivary gland

	Norepinephrine formed in vivo from		
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	[^{14}C]Tyrosine (count/min)	[^{3}H]Dopa (count/min)	In vitro assay of tyrosine hydroxylase*
Decentralized	68	410	3190
Stimulated	358	396	3170

*Tyrosine hydroxylase is expressed as count/min [^{14}C]dopa formed from [^{14}C]tyrosine by an aliquot of homogenate of the salivary glands. (From Sedvall and Kopin, 1967).

Norepinephrine is stored in the nerve terminal in more than one compartment. After the administration of [^{3}H]-norepinephrine, the decrease in its specific activity in tissues was found to be multiphasic (Axelrod et al., 1961), and the specific activity of norepinephrine released by tyramine was dependent on the time the sympathomimetic amine was administered (Potter and Axelrod, 1963b). Kopin et al. (1968) demonstrated that norepinephrine newly synthesized from tyrosine was more rapidly released from the spleen after nerve stimulation. There thus appears to be a relatively small available pool of norepinephrine and a larger reserve store of the catecholamine. The more available pool might be present in the vesicles closest to the synaptic cleft and, because of its location with respect to the neuronal membrane, would be more easily released (Figure 1). The major store of

norepinephrine is located at a greater distance from the neuronal membrane and is thus utilized at a slower rate. This pool might serve as a reservoir for the more readily releasable transmitter.

Axonal transport of catecholamine biosynthetic enzymes

The cell body of the sympathetic neuron is separated from the nerve terminals by long distances (Figure 1). The protein-synthesizing apparatus of the neuron is confined to the cell body, while the terminal is involved in nerve function. The enzymes for the biosynthesis of catecholamines made in the cell body must be transported down the axon to the nerve terminal where most of the neurotransmitter is synthesized. Weiss and Hiscoe (1948) demonstrated that the axon is capable of transporting substances from the cell body to the nerve terminal. Axonal transport is a highly specialized process and different constituents are transported in a proximodistal direction at their own characteristic rate, rapidly (1 to 10 mm/hr) or slowly (1 to 3 mm/day) (Ochs, 1972).

Studies on axoplasmic transport are made by ligation of nerves. When adrenergic axons are pinched, there is a rapid accumulation of norepinephrine and dense core vesicles proximal to the constrictions (Dahlström and Häggendal, 1966). When two ligations are made on the same nerve, no accumulation of norepinephrine is observed above the more distal constriction. Colchicine and vinblastine, compounds that cause a disaggregation of microtubules, block the proximodistal transport of dense core vesicles and norepinephrine in noradrenergic neurons (Hökfelt and Dahlström, 1971) implicating microtubules in the rapid axonal transport.

Biochemical and immunological studies indicate that dopamine-β-hydroxylase (Laduron and Belpaire, 1968) and chromogranins, proteins associated with catecholamine binding, also rapidly accumulate proximal to a constriction in peripheral noradrenergic neurons (Geffen et al., 1969). Recently it has been found that dopamine-β-hydroxylase, an enzyme localized in the storage vesicle, and tyrosine hydroxylase, an enzyme not associated with these vesicles, are both transported down the axon of the rat sciatic nerve at an identical rate (1 to 5 mm/hr) (Coyle and Wooten, 1972). Colchicine blocks the transport of both enzymes. These observations suggest that dopamine-β-hydroxylase and tyrosine hydroxylase are transferred from the cell body to the nerve terminal in close association. Local application of colchicine or vinblastine to the superior cervical ganglion of the rat causes a rapid increase in the levels of dopamine-β-hydroxylase in the ganglia and decrease in the salivary gland (Kopin and Silberstein,

1972). When protein synthesis is inhibited the levels of dopamine-β-hydroxylase in the ganglia are rapidly decreased, indicating that the accumulation of the enzyme is due to new synthesis and the decrease after protein synthesis inhibition is the consequence of transport of the enzyme out of the ganglion. Using this approach, the rate of synthesis of dopamine-β-hydroxylase has been calculated to be 5% of the content per hour.

Release of norepinephrine from nerve terminals

The neurotransmitter, norepinephrine, is contained in a membrane-bound vesicle (Wolfe et al., 1962). Thus its discharge from the nerve after depolarization might occur by release into the cytoplasm followed by rapid passage through the neuronal membrane, or by fusion of vesicular membrane with the neuronal membrane and then liberation, or by an opening of the fused membrane and discharge of norepinephrine into the exterior of the terminal together with the soluble contents of the vesicle. This latter process is called *exocytosis*. Evidence that exocytosis occurs comes from studies with adrenal medulla. Stimulation of the adrenal gland with acetylcholine or electrically results in the release of ATP, as well as catecholamines (Douglas and Rubin, 1961). Acetylcholine can also cause the release of the soluble protein of the chromaffin granule, including dopamine-β-hydroxylase (Viveros et al., 1968). The ratio of norepinephrine to dopamine-β-hydroxylase was found to be the same as that present in the chromaffin granule of the adrenal medulla (Viveros et al., 1969). These findings and microscopic evidence indicate that catecholamines are released from the adrenal medulla by a process of exocytosis. When the sympathetic nerve to the spleen is stimulated, dopamine-β-hydroxylase is released together with norepinephrine (Smith et al., 1970). However, the ratio of the amine to dopamine-β-hydroxylase released was 100 times greater than that found in the vesicles isolated from the splenic nerve. Using a very sensitive assay for dopamine-β-hydroxylase, together with the addition of albumin to protect the enzyme, the ratio of dopamine-β-hydroxylase to norepinephrine released after electrical stimulation of the hypogastric nerve of the vas deferens was found to be similar to that in the soluble portion of the contents of the synaptic vesicle (Weinshilboum et al., 1971e). This data indicates that norepinephrine and dopamine-β-hydroxylase are released from the nerve by a process of exocytosis. The absence of Ca^{++} prevents the release of the enzyme and neurotransmitter, while their discharge is enhanced by increasing the concentration of Ca^{++} to twice that used normally (Johnson et al., 1971). The increased release of dopamine-β-hydroxylase with high Ca^{++} concentration

is blocked by prostaglandin E$_2$. The α-adrenergic blocking agent, phenoxybenzamine, also increases the release of norepinephrine and dopamine-β-hydroxylase in the stimulated vas deferens, but this drug has no effect on the unstimulated preparation. Prostaglandin also blocks the effects of phenoxybenzamine (Johnson et al., 1971). The enhanced release of dopamine-β-hydroxylase by phenoxybenzamine only when the nerve is stimulated suggests that there is an α-adrenergic receptor on the nerve membrane, and blocking this receptor keeps the nerve membrane in a conformational state that allows larger molecules to be secreted for a longer period of time. Prostaglandins may act by interfering with the actions of Ca^{++} and thus reduce the Ca^{++}-dependent secretion of norepinephrine and dopamine-β-hydroxylase.

Microtubules have been shown to be involved in the discharge of intracellular stored products such as the release of ^{131}I from the thyroid gland (Williams and Wolff, 1970), insulin from the beta cells of the pancreas (Lacy et al., 1968), histamine from mast cells (Gillespie et al., 1968), and catecholamines from the adrenal medulla (Poisner and Bernstein, 1971). These findings suggested that microtubules might play a role in the release of dopamine-β-hydroxylase from sympathetic nerve terminals. Treatment of the vas deferens with colchicine and vinblastine, compounds that disaggregate microtubules, almost completely prevented the release of dopamine-β-hydroxylase and norepinephrine when the nerve is stimulated (Thoa et al., 1972). These compounds, how-

ever, have no effect on the spontaneous release of the enzyme or transmitter. Cytochalasin B, a fungal metabolite that disrupts microfilaments (Carter, 1967), also inhibits the release of norepinephrine and dopamine-β-hydroxylase. These findings suggest that both microtubules and microfilaments are involved in release of norepinephrine and dopamine-β-hydroxylase by exocytosis. Microtubules are presumed to function as a cytoskeleton, and nerve depolarization might affect the microtubules in such a way as to direct the vesicles to the proper site on the neuronal membrane where release occurs (Figure 5). Ca^{++} has also been reported to activate the contractile microfilaments in nonmuscle cells (Wessells et al., 1971). These findings would suggest the presence of a contractile microfilament on the neuronal membrane that is activated by Ca^{++}, which makes an opening in the membrane large enough to allow the soluble contents of the vesicle to be released (Figure 5). Cyclic AMP might also be involved since it has been demonstrated that dibutyryl cyclic AMP and theophylline increase the release of norepinephrine and dopamine-β-hydroxylase after nerve stimulation (Wooten, Thoa, Kopin, and Axelrod, unpublished observation).

Circulating dopamine-β-hydroxylase

The observation that dopamine-β-hydroxylase can be released from the adrenal gland and the nerve terminals prompted an examination of the blood for this enzyme.

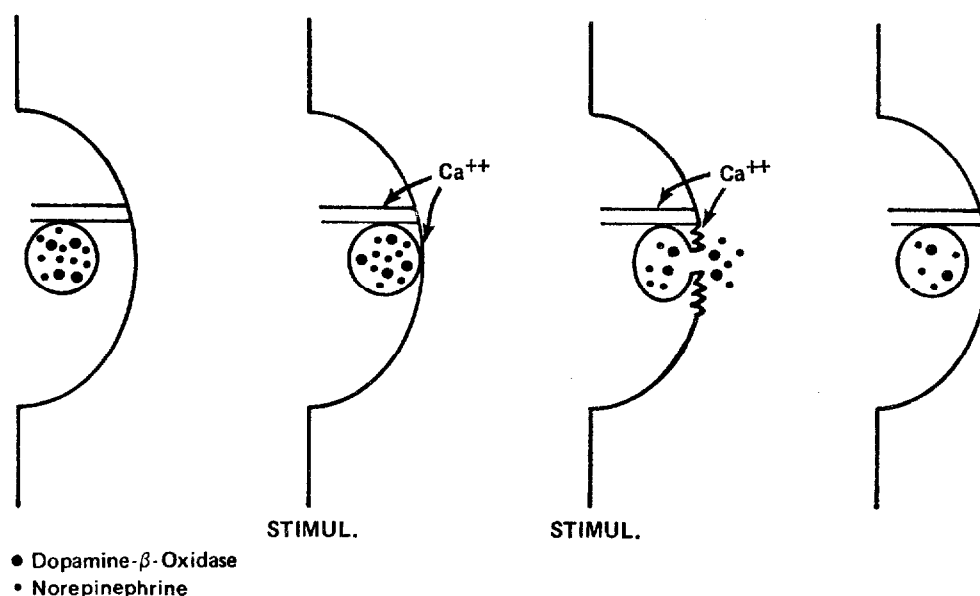STIMUL.   STIMUL.

● Dopamine-β-Oxidase
• Norepinephrine

FIGURE 5   A possible mechanism for release of norepinephrine and dopamine-β-oxidase (hydroxylase) by exocytosis (see text for explanation). (From Axelrod, 1973).

Dopamine-β-hydroxylase was found to be present in the plasma of man and other mammalian species (Weinshilboum and Axelrod, 1971b; Goldstein et al., 1971).The enzyme in theplasma issimilar to purified dopamine β-hydroxylase from the adrenal medulla; both have the same requirements for ascorbic acid, fumarate, and oxygen (Weinshilboum et al., 1971b). They also have similar electrophoretic mobilities and the same Km with respect to substrate.

The plasma dopamine-β-hydroxylase could arise from the sympathetic nerves or the adrenal gland. The administration to rats of 6-hydroxydopamine, a compound that destroys most of the sympathetic nerve terminals but does not affect the adrenal medulla, markedly reduced the level of the plasma dopamine-β-hydroxylase (Weinshilboum and Axelrod, 1971c). On the other hand adrenalectomy did not affect the plasma enzyme levels. The experiments indicate that plasma dopamine-β-hydroxylase comes from sympathetic nerve terminals and that levels of this enzyme in blood suggest a method for measuring activity of these nerves.

In rats subjected to stress, there is an elevation of serum dopamine-β-hydroxylase (Weinshilboum et al., 1971d). When humans were stressed by vigorous exercise or cold pressor test, there was rapid elevation of plasma enzyme. In familial dysautonomia a decrease in plasma dopamine-β-hydroxylase (Weinshilboum and Axelrod, 1971a) is found while subjects with neuroblastoma have an increased enzyme in plasma (Goldstein et al., 1972). Removal of the pituitary gland causes a marked decrease in enzyme activity, which can be prevented by the administration of vasopressin (Lamprecht and Wooten, 1973). This suggests that hypophysectomy, which reduces blood volume, increases sympathetic nerve activity and increases the release of dopamine-β-hydroxylase. Vasopressin increases blood volume and thus results in a reduced sympathetic nerve activity and blood enzyme level.

Regulation of norepinephrine at the neuronal membrane

When norepinephrine is injected into animals it is selectively taken up by sympathetic nerve terminals (Axelrod, 1971; Hertting and Axelrod, 1961). The norepinephrine is then bound in the synaptic vesicle and retained in a physiologically inactive form. This uptake and binding serves as a rapid and effective means of terminating the action of the neurotransmitter. When both monoamine oxidase and catechol-O-methyltransferase, enzymes involved in the metabolism of catecholamines, are inhibited in vivo, the physiological actions

of norepinephrine are only slightly prolonged (Crout, 1961). However, when the uptake of norepinephrine is blocked by drugs (Whitby et al., 1960), or when the sympathetic nerves are destroyed (Hertting et al., 1961b), the response of norepinephrine is considerably increased. These results indicate that uptake of norepinephrine across the neuronal membrane and retention by storage vesicles are a major mechanism for the rapid inactivation of the neurotransmitter (Figure 6).
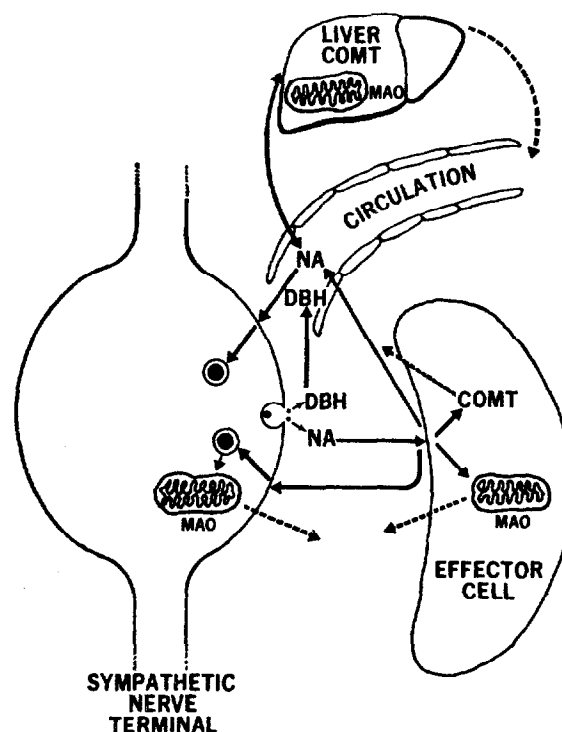

FIGURE 6 Fate[a] of norepinephrine at the sympathetic nerve terminal (see text for explanation). NA is norepinephrine; DBH is dopamine-β-hydroxylase; COMT is catechol-O-methyltransferase; and MAO is monoamine oxidase. (From Axelrod and Weinshilboum, 1972).

The properties of the neuronal uptake mechanism were studied in brain slices (Dengler et al., 1962) and isolated from perfused heart (Iversen, 1963). Uptake of norepinephrine across the neuronal membrane obeys saturation kinetics of the Michaelis-Menten type with high affinity. It also requires sodium ions in the external medium, is temperature dependent, and involves active transport. The uptake process is stereoselective and can be utilized by other phenylethylamine derivatives such as epinephrine, dopamine, tyramine, amphetamine, α-methylnorepinephrine, and meteraminol (Iversen, 1971). High affinity uptake processes similar to norepinephrine's have been demonstrated for other putative neurotransmitters,

serotonin, gamma amino butyric acid, glutamate, aspartate, and glycine (Logan and Snyder, 1971).

Norepinephrine can also be taken up by an extraneuronal process (Iversen, 1965; Eisenfeld et al., 1967). This extraneuronal uptake can be blocked by adrenergic blocking agents, normetanephrine (Eisenfeld et al., 1967) and corticosteroids (Iversen, 1971). Compounds such as isoproterenol, which have a low affinity for the intraneuronal uptake and a high affinity for extraneuronal uptake, may be inactivated by the latter process. Extraneuronal uptake may be an important mechanism for removal of the norepinephrine in which the density of the sympathetic innervation is very low or when the synaptic cleft is wide.

Norepinephrine can be inactivated by a variety of mechanisms (Figure 6): Uptake into the neuron, removal by the circulation, enzymatic O-methylation and deamination by liver and kidney, O-methylation and deamination by effector cells, and extraneuronal uptake. Although neuronal uptake is the major mechanism for terminating the action of the sympathetic neurotransmitter, other types of inactivation may predominate, depending on the density of sympathetic innervation, size of the synaptic cleft, blood supply and activity of catechol-O-methyltransferase, and monoamine oxidase.

Several studies have shown that α-adrenergic blocking agents inhibit the uptake of norepinephrine and cause an increased overflow of the neurotransmitter on nerve stimulation (Hertting et al., 1961b; Brown and Gillespie, 1957). It has also been demonstrated that large amounts of endogenous norepinephrine inhibit the discharge of norepinephrine from nerves (Stärke, 1971). These observations suggest another regulatory site on the neuronal membrane. The neuronal membrane appears to have an inhibitory α-adrenergic receptor (Stärke, 1971), which would cause an increased release of the neurotransmitter when the receptor is blocked and a decreased release when a large amount of norepinephrine is present in the synaptic cleft.

Regulation at the postsynaptic effector cell

The sympathetic effector cell could influence the activity of the sympathetic nerve, and conversely the presynaptic cell could influence the activity of the postsynaptic effector cell. When the response of postsynaptic sympathetic effector cells is blocked by phenoxybenzamine, there is a marked increase in tyrosine hydroxylase activity in the adrenal medulla (Thoenen et al., 1969b) and much greater conversion of [^{14}C]tyrosine to [^{14}C]catecholamine (Dairman and Udenfriend, 1970).

Denervation of the sympathetic nerves leads to an increased response of the postsynaptic cell. One ex-

planation for the increased sensitivity is the removal of an important inactivating mechanism; uptake by the neuronal membrane. Another possible mechanism for supersensitivity is an increased responsiveness of the postsynaptic site. It has been shown that in the denervated muscle, the area of binding of α-bungarotoxin, a compound that binds irreversibly to acetylcholine receptors, is increased (Miledi and Potter, 1971).

The pineal cell has been used to study the relationship between the sympathetic nerves and postsynaptic cell. This gland is richly innervated with sympathetic nerves that regulate the synthesis of the hormone melatonin (Wurtman et al., 1968a). Serotonin N-acetyltransferase is an enzyme that N-acetylates serotonin to form the precursor of melatonin (Weissbach et al., 1960). It is present in the postsynaptic pineal cell and is markedly stimulated by norepinephrine and dibutyryl cyclic 3',5'-adenosine monophosphate in organ culture (Klein et al., 1970). In the intact rat, the enzyme activity is also sharply increased after administration of catecholamines (Figure 7) (Deguchi and Axelrod, 1972). The induction of pineal serotonin N-acetyltransferase by catecholamines is prevented by the β-adrenergic blocking agent, propranolol. When the pineal is denervated the catecholamines cause a superinduction (100-fold increase) of serotonin N-acetyltransferase (Figure 7). Although other possibilities have not been excluded, these findings suggest that the increased responsiveness after denervation is due to changes on the postsynaptic β-adrenergic receptor on the pineal cell.

Conclusions

The formation and conservation of the sympathetic neurotransmitter, norepinephrine, is controlled at several sites in the sympathetic neuron. Its synthesis can be rapidly changed by feedback mechanisms on tyrosine hydroxylase in the nerve terminals. Another regulatory site occurs in the cell body and adrenal medulla whereby sympathetic nerve activity changes the rate of formation of various biosynthetic enzymes. Glucocorticoid hormones also influence the synthesis of these enzymes in the adrenal medulla. The biosynthetic enzymes are made in the cell body and transported down the axon to the nerve terminals via proximodistal flow. The transmitter is then synthesized and stored in a vesicle in the nerve terminal. Norepinephrine is released by a process of exocytosis. Once released, the neurotransmitter can be taken up by the nerve terminal and stored and reused again. The presynaptic membrane can affect the activity of the postsynaptic cell by rapidly removing the transmitter by reuptake and influencing the responsiveness of the postsynaptic adrenergic receptor.
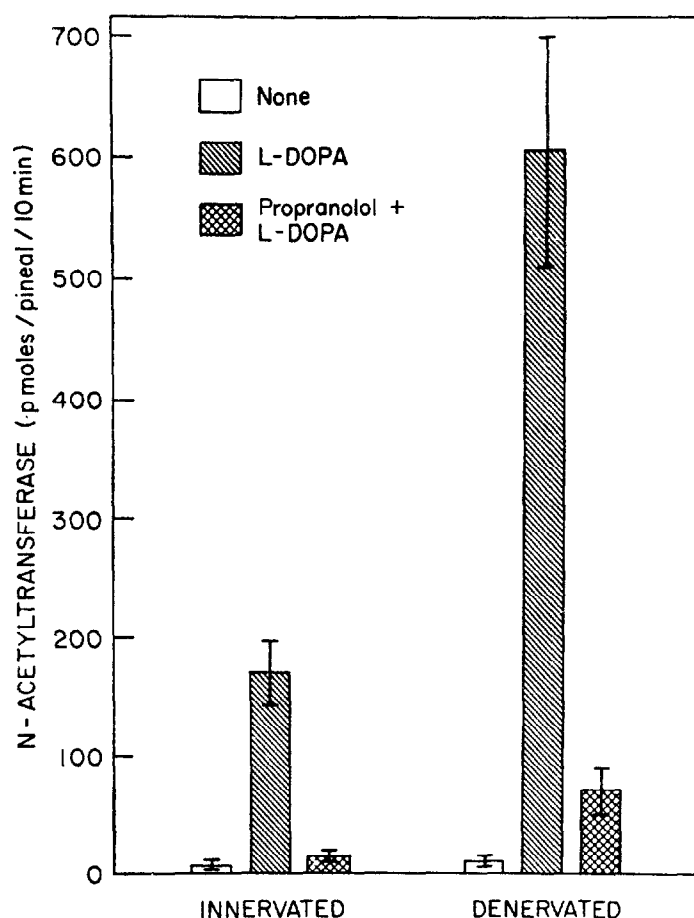
FIGURE 7 Induction and superinduction of serotonin N-acetyltransferase in the rat pineal. Rat pineals were denervated by the bilateral removal of the superior cervical ganglia. Dopa (150 mg/kg) alone or together with propanolol (20 µg/kg) were given at 10 AM and N-acetyltransferase in the pineal was examined 3 hrs later (Deguchi and Axelrod, 1972).

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